Peer Review
Experimental Toxicology Division
NHEERL

PARTICULATE MATTER

DISEASE MODELS TO INVESTIGATE MECHANISMS AND SUSCEPTIBILITY TO PM-INDUCED CARDIOVASCULAR AND PULMONARY HEALTH EFFECTS

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INTRODUCTION

- We eat three times a day, we drink ten times a day, but we breath thousands of times a day, so it is logical to believe that a small degree of air pollution can have a significant impact on health, given that the large airway surface is continuously exposed pollutants.
- A complex array of toxic PM components can have access to a variety of cells and organs.
- Frail individuals with diseases may have no compensatory reserve to counteract the effects of PM.
- Genetic and environmental factors can modulate variation in susceptibility to air pollutants.
- The challenge is to provide causative evidence of cellular and molecular events through animal experimentation using realistic exposure scenarios.

PROGRAM GOALS

- Identify source-specific causative constituents of PM using compositionally-different PM surrogates
- Investigate potential mechanisms underlying susceptibility to PM-induced cardiovascular and pulmonary health effects
- Employ novel approaches to study cardiovascular impact from pulmonary exposure
- Identify genetic and environmental risk factors of susceptibility using rat models of human diseases
- Develop a rat model of human relevant Chronic Obstructive Pulmonary Disease (COPD)

APROACHES

- Use multi-disciplinary collaborative approach in addressing above mentioned goals.
- Employ genetically-predisposed and experimentally-induced animal models of human cardiopulmonary diseases
- Use compositionally-dissimilar surrogate combustion or synthetic as well as ambient PM
- Use disease intervention to identify the role of genetic versus physiologic risk factors in susceptibility
- Investigate mechanisms using molecular approaches to identify the roles of oxidative stress and microvascular thrombosis in cell signaling and gene expression leading to pathologenesis

METHODS

Types of air pollutants: Fine residual oil combustion PM (ROFAs) of varied metal composition, individual metals (iron, vanadium, nickel and zinc), fine and ultrafine carbon, synthetic fine PM, collected ambient PM, and real time ambient particles (CAPs)

Animal strains and models: Male Sprague Dawley (SD), Brown Norway, Wistar-Kyoto, Spontaneously Hypertensive rats; rats with experimentally-induced pulmonary diseases (SO2-induced COPD/bronchitis, monocrotaline-induced pulmonary hypertension; *in vitro* cultured macrophages and pulmonary fibroblasts

Exposure methods and durations: Whole-body inhalation, nose-only inhalation, intratracheal instillation (IT) of suspended PM; *in vitro* exposure of cells. Exposure periods range from single acute to up to 16 week exposures

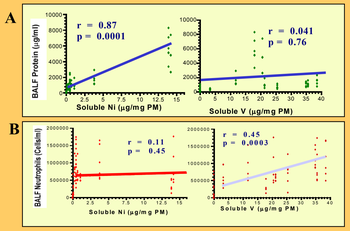
Endpoints: BAL markers of pulmonary injury/inflammation, histomorphometric evaluation, pulmonary and cardiac physiologic evaluation, measurements of cell signaling proteins, nuclear factors and gene expressions using Western blotting, immunohistochemistry, PCR, real time PCR, gel shift assays and gene arrays

MECHANISMS: OXIDATIVE STRESS AND METALS

Biological networking of cardiovascular and pulmonary systems

Metal-Specific mechanisms

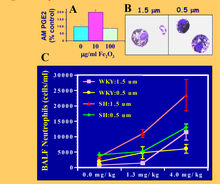
PM-associated nickel was significantly correlated with pulmonary vascular leakage (A) and vanadium was correlated with neutrophilic inflammation (B) 24 h following exposure



Note: Rats were intratracheally instilled with saline or one of 10 compositionally-different oil combustion PM at three concentrations. Twenty-four hrs later, pulmonary vascular leakage and inflammation were quantified. Data were analyzed using multivariate regression models to assess the causative role of soluble metal constituents in pulmonary response.

Potential anti-inflammatory effects of soluble iron

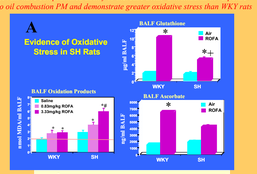
We have demonstrated that ~1% of iron is leachable/d from Fe_2O_3 particles within alveolar macrophage, however, leached iron is not released out of cells. Phagocytosis of Fe_2O_3 particles causes increased PGE2 production but not inflammatory cytokine release suggesting that soluble iron within AM may suppress phagocytosis-induced activation of AM by production of anti-inflammatory proteins. Consequently, larger Fe_2O_3 particles caused more inflammatory response than smaller particles which perhaps can be attributable to a smaller release of iron from larger particles. This possibility is tested by analysis of PGE2 using both ice particles.



A. WKY rat alveolar macrophages were incubated with 1.5 µm Fe_2O_3 particles for 24 h and cellular PGE2 levels were analyzed. B. *In vivo* Fe_2O_3 particle phagocytosis by AM. Note the particle size. C. Inflammatory response in rats after instillation of Fe_2O_3 particles of two different sizes.

PM-induced oxidative stress in SH rats

SH rats have compromised ability to increase BALF antioxidants in response to oil combustion PM and demonstrate greater oxidative stress than WKY rats

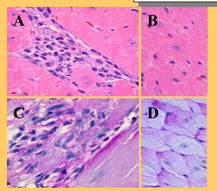


Notes: In study A, rats were exposed nose-only to Florida power plant fugitive ROFA 15 mg/m3x6h/dx3d and BALF glutathione, ascorbate, and TBA reactive materials were analyzed 18 h post-exposure. In study B (upper penal), rats were exposed either intratracheally and responses determined 1, 2, or 4 days post or (lower penal) exposed nose-only to 15 mg/m3x6h/dx3/wk for 1, 2 or 4 weeks and responses analyzed 1 or day post. Missing bars indicate no rats were exposed for that time point.

Myocardial injury from long-term PM exposure

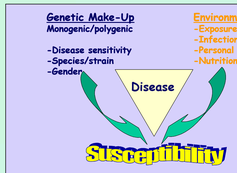
Atmospheric particles from Ottawa, Canada, Utah Valley, USA, Mexico City, Mexico, Lodz,Poland, and New Delhi, India contain high concentration of zinc. We have demonstrated myocardial lesions in WKY rats following episodic inhalation of fugitive combustion PM containing zinc. Zinc concentration in this sample was similar to zinc levels in Ottawa and Lodz PM.

Element	Water-leachable µg/mg	1 M HCl leachable µg/mg
SO_4	107.0	134.9
Zn	14.5	14.6
Ni	3.0	3.2
Fe	2.5	65.4
V	0.1	15.4
Co	0.1	0.1
Cu	0.1	0.2
Mn	0.4	0.5



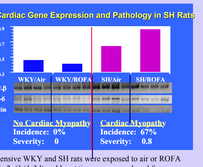
Note: SD, WKY and SH rats were exposed to fugitive combustion PM collected off the stack of a power plant burning residual oil. Rats were exposed nose-only at 10 mg/m3x6h/dx1d/wkx16 wks and myocardial lesions were characterized using a variety of staining techniques.

SUSCEPTIBILITY



SH rat, a model of cardiovascular disease

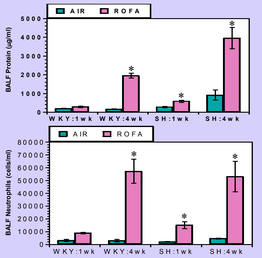
In a series of studies conducted over the past 6-7 years, we have provided evidence that SH rats indeed have underlying cardiac disease. Phenotype of hypercoagulability, systemic oxidative stress, systemic inflammation (presence of activated granulocytes in the blood), borderline pulmonary hypertension, and vulnerability to infections matches to human cardiovascular disease patients



Normotensive WKY and SH rats were exposed to air or ROFA (15 mg/m2x6h/dx3d) and heart tissues were analyzed for inflammatory gene expression using PCR. Note that underlying cardiac disease is associated with increased inflammatory baseline gene expression in SH rats when compared to WKY rats.

Progressive lung injury with continued PM exposure

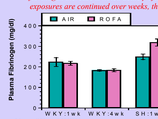
We have demonstrated that oil combustion PM-induced lung injury, unlike injury induced by ozone, is progressive over time when episodic inhalation exposures last for weeks



Note: SH and WKY rats were exposed nose-only to oil combustion PM for 6h/d3d/wkx1, 2, or 4 consecutive weeks. Rats were sacrificed either 1 or 4 day post-exposure and and BALF neutrophils were quantified. These values are pooled for 1- and 4-day time points because similar responses were noted for both time points.

Plasma fibrinogen: An acute response to PM

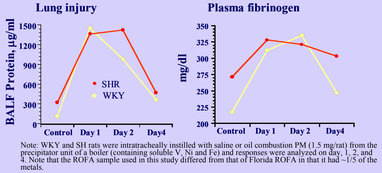
Either with intratracheal or with nose-only inhalation, plasma fibrinogen increase is noted acutely after PM exposure. If the exposures are continued over weeks, this response disappears.



Note: SH and WKY rats were exposed nose-only to oil combustion PM for 6hx6x3d/wkx1, 2, or 4 consecutive weeks. Rats were sacrificed either 1 or 4 days post-exposure and plasma fibrinogen levels were measured. These values are pooled for 1- and 4-day time points.

Relationship between plasma fibrinogen levels and lung injury

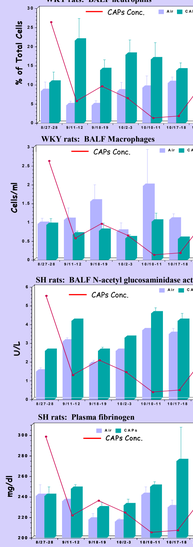
Temporal relationship existed between lung injury/inflammation and plasma fibrinogen increase following intratracheal exposure of rats to oil combustion PM



Note: WKY and SH rats were intratracheally instilled with saline or oil combustion PM (1.5 mg/rat) from the precipitator unit of a boiler (containing soluble V, Ni and Fe) and responses were analyzed on day. 1, 2, and 4. Note that the ROFA sample used in this study differed from that of Florida ROFA in that it had ~1/5 of the metals.

Consistent CAPs effects in repetitive studies

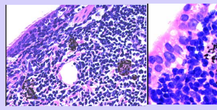
Acute RTP CAPs exposures during August –October, 2001 provided rat strain specific consistent pulmonary and vascular responses. WKY rats demonstrated marked increases in neutrophils and decreases in lavageable macrophages in each CAPs exposure study. SH rats demonstrated increased BALF N-acetyl glucosaminidase activity (marker for macrophage activation) and plasma fibrinogen increase in response to CAPs exposure.



Note: WKY or SH rats were exposed to filtered air or RTP CAPs during August-October, 2001, 4h/d for 2 consecutive days and Responses were determined 1 d post. Note that on September 11, 2001, no exposures occurred, so this study involved exposure for only

Particle translocation to lung-associated lymph nodes

We have noted that particle-laden macrophages migrate to the lung-associated lymph nodes, especially when episodic inhalation exposures occur over weeks. Of particular interest was that SH rats seem to have more prominent lymph nodes and more particle-loaded cells than WKY or SD rats.

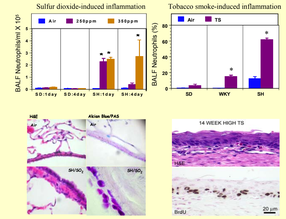


Note: SH rats were exposed nose-only to oil combustion PM10 mg/m3, 6h/dx1d/wkx16 wks. H&E sections of lung associated lymph nodes showing particle laden macrophages.

DEVELOPING A RAT MODEL OF COPD

Sensitivity of SH rats to experimental induction of pulmonary disease: Developing a rat model of COPD

Lack of sensitivity of existing animal COPD models to PM exacerbation of pulmonary injury prompted us to examine the validity of these models for their relevancy to human disease. Unlike chronic active inflammation and airways disease of smoker COPD patients, experimental exposure of conventional laboratory rats to cigarette smoke and sulfur dioxide demonstrated only a modest and rapidly reversible inflammation and pulmonary disease. We hypothesized that genetic predisposition is necessary for developing human-like COPD in rats, and employed SH rats in experimental induction of sulfur dioxide and tobacco smoke-induced pulmonary disease. SH rats demonstrated remarkable sensitivity to inflammation and airway mucus cell hyperplasia with tobacco smoke and sulfur dioxide exposure. Although the precise genetic markers are yet to be identified, we know that SH rats demonstrate similar phenotypic risk factors as those present in COPD patients, and believe that there are common susceptibility traits that predispose rats to the disease. In addition to the utility of this new COPD model in air pollution studies, this model will provide the opportunity and a tool for understanding the role of genetics in susceptibility to a disease and an environmental insult such as PM exposure.



Male SD, and SH rats were exposed to sulfur dioxide 5h/d/ 4d (BALF neutrophils) or 7 d (4d/wk1 and 3d/wk2) and lung tissues were examined histologically.

Male SD, WKY and SH rats were exposed to tobacco smoke (TS), ~70-80 mg/m3, 6h/dx2 d (BALF neutrophils), or 3d/wkx14 wks (histology). BrdU was injected i.p., prior to necropsy and proliferating airway cells were examined immunohistochemically.

Conclusions and Impact

- Our studies highlight consideration of metal-specific mechanisms, contribution of oxidative stress in susceptibility, and the role of microvascular thrombosis in cardiovascular injury, and thus, support epidemiological findings, and show that specific PM sources may have unique health outcomes. Studies conducted under goal 8 program involve the role of genetic predisposition to oxidative stress and identification of genetic markers in susceptibility to PM components and other air pollutants. Studies using CAPs and other combustion source surrogate PM point to the susceptibility of individuals with cardiovascular diseases, and that unique approaches are needed to study cardiotoxicity of inhaled pollutants. We also provide evidence that animal models with genetic disease predisposition may be responsive and highly relevant in PM health studies.
- Research publications on the metal-specific mechanisms, effects of CAPs on bronchitic and SH rats, introduction of SH rats in PM studies, role of predisposition to oxidative stress in susceptibility to PM, development of COPD model, and first demonstration of myocardial injury with zinc-containing PM have impacted the PM research program by providing needed information in consideration of new PM standards. This research program includes realistic exposures spanning a wide range of PM concentrations, involves use of novel pathobiologic and molecular approaches, and is well recognized.

Future Directions

- Investigate mechanisms of zinc and ultrafine carbon-induced cardiovascular injury.
- Use interventions to modify disease status prior to PM exposure for identification of cardiovascular risk-factors.
- Investigate the role of microvscular thrombosis in systemic and cardiotoxic effects of fine/ultrafine PM and metals.
- Identify mechanisms by which oxidative stress and endothelial inflammation impact blood coagulation.
- Develop approaches to investigate the role of PM constituent-specific mechanisms of mitochondrial oxidative stress.
- Further characterization of sulfur dioxide and tobacco smoke-induced pulmonary disease in SH rats.
- Identification of genetic markers modulating oxidative stress and linking to susceptibility.

SOLVING AGENCY PROBLEMS